Thermal Resistance of *Salmonella* spp. and *Listeria monocytogenes* in Liquid Egg Yolk and Egg Yolk Products†

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ABSTRACT

The effectiveness of various pasteurization procedures in destroying *Listeria monocytogenes* and *Salmonella enteritidis* in liquid egg products was evaluated. Survivor studies were performed on individual strains of *L. monocytogenes* and *L. innocua* in commercially broken raw egg yolk samples after heating at 61.1, 63.3, and 64.4°C using submerged vials, and on *Salmonella* spp. at 60.0, 61.1, and 62.2°C. Surviving bacteria were enumerated on TSA and results expressed as D-values. The influence of a_w-lowering ingredients such as salt and sugar on thermal resistance in yolk was investigated using a five-strain mixture of *L. monocytogenes* or a mixture of *Salmonella* spp. (four strains of *S. enteritidis*, one strain each of *S. senftenberg* and *S. typhimurium*) at 61.1°C to 66.7°C. At 61.1°C (present minimum temperature for pasteurization of plain egg yolk), a 7-log-unit reduction of *Salmonella* took 1.4 to 2.4 min, whereas a 7-log-unit reduction of *L. monocytogenes* took 4.9 to 16.1 min. The D-value for *L. monocytogenes* at 64.4°C increased from 0.44 min in plain yolk to 8.26 min after a 21.5-min lag (total time to achieve 1-log-unit reduction was 30.7 min) in yolk with 10% salt and 5% sugar, and 27.3 min after a 10.5-min lag (total time 37.8 min for 1-log-unit reduction) in yolk with 20% salt. The D-value for *Salmonella* in egg yolk at 64.4°C was < 0.2 min, but when 10% salt was added, the D-value was 6.4 min. A_w-lowering solutes in liquid egg yolk increased the thermal resistance of *Salmonella* and *L. monocytogenes*.

Key words: egg yolk, heat resistance, pathogens, *Salmonella*, *L. monocytogenes*

Both *Salmonella enteritidis* and *Listeria monocytogenes* have assumed increased importance recently in shell eggs and egg products. Over the past several years there has been a sharp rise in *S. enteritidis* foodborne illness in the United States and Europe. A majority of these salmonellosis outbreaks have been associated with consumption of raw or lightly cooked, *S. enteritidis*-contaminated eggs, and health officials are concerned that the incidence may continue to increase *(4, 24)*. The microorganism is capable of growth in intact shell eggs at temperatures ≥10°C *(12)*. While there have been no documented outbreaks of listeriosis associated with eggs, this pathogen is of concern because of its ability to grow in refrigerated whole egg *(5)* and egg yolk *(22)*. *L. monocytogenes* has been isolated from commercial raw liquid whole egg *(13, 16)*. Further, the thermal resistance of *L. monocytogenes* in liquid whole egg is considerably greater than that of *Salmonella* species, with the exception of *S. senftenberg* 775W *(5, 6)*.

Increased convenience along with concern over the presence of *Salmonella* in shell eggs has prompted increased use of pasteurized liquid egg products and the introduction of a variety of new liquid egg products. This prompted the USDA Agricultural Marketing Service to establish a cooperative research program to better define the processing requirements needed for the thermal inactivation of *S. enteritidis* and *L. monocytogenes*. The current study focuses on two of the attributes investigated, the heat resistance of *S. enteritidis* and *L. monocytogenes* in egg yolk, and the effect of a_w-lowering solutes. Solutes such as sodium chloride and sucrose have been reported to increase the thermal resistance of heat-sensitive *Salmonella (2, 9, 25)*. Ng et al. *(18)* demonstrated increased heat resistance of *Salmonella* and *Arizona* in salted whole egg, and Garibaldi *(7)* reported increased heat resistance in both salted and sugared egg yolk. Likewise, reduction in $_{aw}$ from 0.98 to 0.90 by the addition of sucrose increased the heat resistance of *L. monocytogenes* 10-fold *(25)*.

MATERIALS AND METHODS

Cultures

The origin of the *S. enteritidis* strains used in this study are as follows: strain 2000 from I. Walls (Eastern Regional Research Center, U.S. Department of Agriculture); and strains 5-19 (iso-

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lated from yolk sac of a dead chick), strain Y8P2 (isolated from yolk), and 92-008 (environmental isolate) from Charles Benson (Univ. of Penn. School of Veterinary Medicine). *S. senftenberg* Pro 168 Grp was obtained from C. Benson, and *S. typhimurium* was obtained from I. Walls. Cultures were maintained individually in tubes of tryptose phosphate broth (TPB) (Difco Laboratories, Detroit, MI) at 5°C. For heating studies, the strains were grown individually in 50 ml of TPB in a 250-ml Erlenmeyer flask on a rotary shaker (37°C, 22 h, 150 rpm). A cocktail of *Salmonella* spp. was prepared by blending equal volumes of each culture immediately before use. The origins of the *L. monocytogenes* strains are as follows: Scott A (clinical isolate, USDA, originally from J. Hunt, FDA, Cincinnati), 2284 (isolated from chicken breast meat) and Scott A 2045 (both from Sharon Franklin, ARS, Ames, IA), ST.L. and V-7 (Edward Hoerning, USDA, Gastonia, NC). Cultures were carried individually in tubes of brain heart infusion (BHI) broth (Difco) at 5°C. *L. innocua* 2430, isolated from commercial raw egg, was obtained from S. Franklin. For heating studies, each strain was grown individually in 50 ml of BHI with 0.3 grams/l of additional glucose in a 250-ml Erlenmeyer flask on a rotary shaker (37°C, 24 h, 150 rpm). Five-strain mixtures were prepared by combining equal volumes of *L. monocytogenes* cultures immediately before use.

Heating menstruum

Commercially broken raw egg yolk was obtained from local egg processors. For sealed-vial studies, product was shipped cold but not frozen in sterile 8-oz. containers and arrived at the laboratory within 24 h of breaking of the eggs. Aerobic plate counts were determined on each batch of yolk received, as described below. Salt (NaCl) and/or sugar (sucrose) from a local market were added immediately before use. Survival curves were determined in egg yolk, yolk containing 10% (wt/wt) salt, 20% salt, 10% sugar, and 10% salt plus 5% sugar. Survival of the background flora was determined by the same procedure as used for inoculated samples. Unpasteurized yolk for plate pasteurization was obtained in 30-lb. pails within 2 h of use.

Water activity

The water activity (a_w) of each heating menstruum was measured in triplicate with a Decagon CX-2 water activity meter (Decagon Devices, Inc., Pullman, WA) at 22 ± 0.5°C.

Heating procedure

In the initial experiments, raw egg yolk was inoculated with individual strains of *Salmonella* or *Listeria*. Five-strain mixtures of *L. monocytogenes* and a cocktail of the *Salmonella* spp. were used in place of individual strains in subsequent experiments. Samples were inoculated to give $\log_{10}$ counts of 8.5 to 9.0 in unheated product. This ensured that the pathogen being tested greatly outnumbered the natural background flora, allowing enumeration on nonselective media with inconsequential interference by the background flora.

Yolk or yolk with additives (4.5 g) was weighed into a 9-ml glass vial (15-mm OD by 60-mm height), 0.5 ml of culture added, and the vial closed with a rubber septum and plastic lid. The contents were then mixed, tempered to 25°C, and submerged in a controlled-temperature water bath (Lauda, model MS-20; GMBH & Co. KG, Konigshofen, Germany). Temperature was monitored with a thermocouple (type T) inserted into the geometric center of a control vial containing 5 g of uninoculated egg yolk. The temperature was recorded with an Omega Recorder, Series RD-2000 (Omega Engineering, Inc., Stamford, CT). Timing began when the temperature of the control vial reached the experimental temperature. At intervals appropriate for the temperature, vials were removed from the heating bath and cooled immediately in a slush ice bath. To determine survival of the background flora, 0.5 ml of peptone water was added in place of culture.

For plate pasteurization studies, yolk was inoculated with single-strain cultures of *S. senftenberg* or *L. innocua* at the same level as in sealed vial studies. An APV Crepaco Junior Plate Pasteurizer (Crepaco, Chicago, IL) was used to heat inoculated yolk at 61.1, 63.3, and 64.4°C for 3.5 min. The temperature was measured at the end of the holding tube. Samples of unheated and heated products were immediately placed in an ice bath. Three trials were conducted for each organism; each temperature was used in each trial. Replicates at each temperature represent different batches of egg and culture.

Bacteriology

The number of surviving microorganisms was determined by surface plating onto tryptic soy agar (Difco) using a Spiral plater (Model D; Spiral Biotech, Bethesda, MD). The cooled egg yolk (1.1 g) was weighed into sterile tubes, 9.9 ml of sterile 0.1% peptone (Difco) water added and the mixture shaken. Further dilutions were prepared in peptone water as needed. Colonies of surviving *Salmonella* were counted after 24 h at 37°C; surviving *Listeria* were counted after 48 h at 37°C. Aerobic plate count was determined by the same procedure on each batch of egg yolk received prior to inoculation.

Data analysis

Counts were transformed into $\log_{10}$ values. The data were then analyzed by linear regression using a commercial spreadsheet program. D-values were calculated as the negative reciprocal of the slope. In a few instances (*L. monocytogenes* in products with added salt), where the decrease in numbers was slow and a distinct shoulder was observed, the nonlinear inactivation model of Whiting *(26)* was used. The correlation coefficient (r^2) in all cases was 0.8 or above (most were over 0.95), indicating that the survivor plots (log number versus time) were linear, and D values could be calculated from the slopes of the lines. All D-values reported are averages of three trials. D-values were transformed into $\log_{10}$ values and plotted against temperature; z-values were calculated as the negative reciprocal of the line. Regression coefficients for these plots are reported. For pasteurization experiments, the reduction in viable count was calculated.

RESULTS AND DISCUSSION

The background count of 120 samples of raw egg yolk used for sealed-vial studies was 4.41 ± 0.74 CFU/g. Survival experiments on uninoculated yolk and yolk with added sugar or salt indicated that most of the background flora died during the come-up time. Surviving organisms were not visible when plates were counted at 24 h, but colonies appeared at 48 h in low numbers (2.3 to 3.0 log CFU/g) which were constant up to 5 min of heating time. Therefore no interference of background flora with *Salmonella* counts occurred; counts of less than 3.0 log CFU/g occurred only as the last one in 8 to 10 or more points on the survival curve for *L. monocytogenes* (Fig. 2) and would not increase the calculated D-value. No tailing that could be attributed to heat-resistant background flora was observed in any experiment. D- and z-values for the individual *Salmonella* strains are shown in Table 1. Differences between the individual *S. enteritidis* strains and

between *S. enteritidis* and two other species commonly found in egg were relatively small. Shah et al. *(21)* determined D-values for 17 strains of *S. enteritidis* in liquid whole egg at 60°C and found values ranging from 11.8 to 31.3 s (0.20 to 0.52 min). The heat resistance of *Salmonella* is known to be higher in yolk than in whole egg; Garibaldi et al. *(8)*, in a study of heat-resistance characteristics of *S. typhimurium* TM-1, obtained D-values at 60°C of 0.27 min for whole egg and 0.4 min for egg yolk. Ng *(17)* compared the heat sensitivity of 300 cultures of salmonellae to *S. typhimurium* TM-1 at 57°C at pH 6.8 in trypticase soy medium supplemented with 1% yeast extract. The ratio of the heat resistance of each strain to that of *S. typhimurium* TM-1 was calculated. The most heat-resistant *S. typhimurium* was 1.69 times as resistant as TM-1; the mean value of this ratio was 1.24. For *S. senftenberg* (excluding 775W), the most resistant strain was 1.80 times as resistant as *S. typhimurium* TM-1; the mean value of this ratio for *S. senftenberg* strains was 1.15. The average of all 300 strains tested was 1.2 times that of TM-1. The heat resistance of our strains appears to be within the range predicted from the above data and should represent the more heat-resistant of the strains commonly found in egg products. In subsequent sealed-vial studies, a mixture of these six strains was used.

D- and z-values for individual strains of *L. monocytogenes* and *L. innocua* are shown in Table 2. More variability was observed between strains of *L. monocytogenes* than between strains of *S. enteritidis*. Sorqvist *(23)* studied the heat resistance of different serovars; at 60°C in physiological saline, D-values ranged from 0.61 to 2.9 min. Foegeding and Leasor *(5)* obtained values of 1.3 to 1.7 min at 60°C in liquid whole egg. McKenna et al. (15) reported D values of 55.1 to 101.3 s (0.92 to 1.69 min) at 60.0°C and 35.7 to 57.1 s (0.60 to 0.95 min) at 62.8°C in whole egg. Harrison and Huang *(10)* studied the thermal resistance of *L. monocytogenes* Scott A in crabmeat; when heated cells were recovered on trypticase soy agar, a D-value of 2.61 min at 60°C and a z-value of 8.40°C were obtained. Holsinger et al. *(11)* determined the heat resistance of *L. monocytogenes* Scott A in phosphate buffer, homogenized milk, and nine ice cream mixes. At 60°C, a D-value of 2.10 min was obtained in homogenized milk and 1.14 min in buffer; in ice cream mixes, D-values ranged from 2.21 to 4.79 min (calculated by linear regression) after a lag period of 2.04 to 5.24 min. Schoeni et al. *(20)* used a five-strain mixture of *L. monocytogenes* to determine rates of inactivation in beef and fermented beaker sausage. When cells were recovered on tryptose agar, the D-values at 60°C were 4.47 ± 1.60 min in ground beef roast and 9.13 ± 2.50 min in fermented beaker sausage. At 62.8°C in ground beef roast, the D-value obtained was 2.56 ± 1.04 min. Bhaduri et al. *(3)* studied the thermal destruction of *L. monocytogenes* Scott A in liver sausage slurry. D-values were 8.91 min at 57.2°C, 2.42 min at 60°C, and 1.12 at 62.8°C; the z value was

TABLE 1. *Thermal resistance of* Salmonella *strains in egg yolk at three temperatures*

Strain[a]	D-values (min) 60.0°C	61.1°C	62.2°C	z-values (°C)	Regression coefficient (r^2)
SE 2000	0.62 ± 0.08[b]	0.27 ± 0.01	0.23 ± 0.03[c]	5.11	0.868
SE 5-19	0.65 ± 0.09	0.29 ± 0.05	0.30 ± 0.09[c]	6.55	0.717
SE Y8-P2	0.55 ± 0.12	0.27 ± 0.07	0.21 ± 0.04[c]	5.26	0.929
SE 92-008	0.75 ± 0.06	0.35 ± 0.03	0.25 ± 0.03[c]	4.61	0.952
SS	0.73 ± 0.12	0.28 ± 0.03	0.21 ± 0.12[c]	4.07	0.912
ST	0.67 ± 0.21	0.20 ± 0.23[c]	0.14 ± 0.16[c]	3.24	0.910

[a] SE, *Salmonella enteritidis*; SS, *S. senftenberg*; ST, *S. typhimurium*.
[b] Average of 3 replicates ± standard deviation.
[c] Estimated (see text).

TABLE 2. *Thermal resistance of* L. monocytogenes *(LM) and* L. innocua *(LI) strains in egg yolk*

Strain[a]	D-values (min) 61.1°C	63.3 °C	64.4°C	z-values (°C)	Regression coefficient (r^2)
LM Scott A	1.56 ± 0.23[a]	0.92 ± 0.14	0.82 ± 0.14[b]	11.45	0.973
LM 2284	2.30 ± 0.40	1.28 ± 0.53	0.66 ± 0.25	6.35	0.950
LM ST.L.	0.92 ± 0.11	0.38 ± 0.07	0.46 ± 0.06[b]	9.70	0.735
LM Scott A 2045	0.94 ± 0.12	0.35 ± 0.05	0.21[c]	5.08	0.99993
LM V-7	0.70 ± 0.48	0.43 ± 0.13	0.19 ± 0.22	6.22	0.892
LI 2430	2.29 ± 0.16	1.12 ± 0.11	0.69 ± 0.18	6.43	0.994

[a] Average of 3 replicates ± standard deviation.
[b] Estimated (see text).
[c] Based on one trial. In two trials, a 6-log-unit reduction occurred during come-up time; insufficient viable cells remained to determine D-value.

6.20°C. Mackey et al. *(14)* studied the heat resistance of an isolate of *L. monocytogenes* from prepackaged chicken inoculated into chicken leg, chicken breast, and beef. D-values at 60°C were 5.6, 8.7, and 3.8 min, respectively. Although direct comparisons cannot be made between our data and those in the literature because of differences in heating menstruum and temperature, the thermal resistance of our strains appears to be consistent with these literature values. The thermal resistance of the *L. innocua* strain used was similar to that of the most resistant *L. monocytogenes*, and was used in plate-pasteurization studies. For subsequent sealed-vial studies, a mixture of *L. monocytogenes* strains was used.

Some inconsistencies occurred at the highest temperatures with both genera of bacteria. Considerable destruction occurred during the come-up time, and D-values are based on cells that survive the come-up period. Destruction was rapid, and the number of data points obtained was smaller than at lower temperatures. Therefore the slope of the survival curve at the highest temperature is influenced to a greater extent by the greater heat resistance of the last survivors. For example, at 62.2°C with strain SE 5-19, viable numbers were decreased by 1.6 to 2.9 log cycles during the come-up time and complete destruction occurred within 2 min. For *L. monocytogenes* Scott A 2045 at 64.4°C, almost no cells survived the come-up time in two of the three runs. For *L. monocytogenes* ST.L. at 64.4°C, a 2.9 to 4.1-log-cycle reduction occurred during the come-up time. Use of the Spiral Plater, which was necessitated by the large volume of plating performed in this study, required a 1:10 dilution of egg yolk. For accuracy, at least 20 colonies per plate should be present. This represents a viable count in the heated egg yolk of log 3.62 cells per g. Because of the rapid killing during the come-up time, most of the plate count data used in calculating the D-values for the more heat-sensitive strains at the higher temperatures are at or below the recommended level of 20 colonies. Therefore these values should be regarded as estimates, as noted in Tables 1 and 2.

Destruction by plate pasteurization is compared to reduction in numbers calculated from sealed-vial data in Table 3. For *L. innocua*, good agreement between the two techniques was obtained. In the case of *S. senftenberg*, no survivors were found after heating at the higher temperatures; at 61.1 °C, surviving background flora made it impossible to determine *Salmonella* survivors without the use of selective media. Selective media were not used in these experiments to maximize recovery of injured cells.

Examples of survivor curves for *Salmonella* heated at 63.3°C in egg yolk and egg yolk with a_w-modifying solutes are shown in Figure 1. The protective effect of sugar, salt, and a salt-sugar combination is apparent. Survival of *L. monocytogenes* in the various egg yolk formulations during heating at 64.4°C is shown in Figure 2. A substantial lag period before initiation of destruction was observed with the samples containing salt. Mackey et al. *(14)* observed an increased lag before destruction of *L. monocytogenes* when beef fat and curing salts were added to minced beef. Holsinger et al. *(11)* observed lags of 2.04 to 5.24 min at 60°C before viable numbers of *L. monocytogenes* declined in ice cream mixes. Bhaduri et al. *(3)* observed both a shoulder and tailing on survivor curves of the Scott A strain in liver sausage slurry.

TABLE 3. *Log-unit reductions of* S. senftenberg *and* L. innocua *in egg yolk after 3.5 min of heating at indicated temperatures achieved with actual plate pasteurization (average of three trials) compared to reduction calculated from D-values from sealed-vial study*

		Temperature (°C)		
Species	Method	61.1	63.3	64.4
S. senftenberg	Vial	12.5	ND	ND
S. senftenberg	Plate	≥ 4.95[a]	≥ 8.32[b]	≥ 8.75[c]
L. innocua	Vial	1.53	3.11	5.09
L. innocua	Plate	1.47	3.34	4.05

[a] Interference by surviving background flora on nonselective medium.
[b] In 2 of 3 trials, no survivors were found.
[c] No survivors were found in any trial.

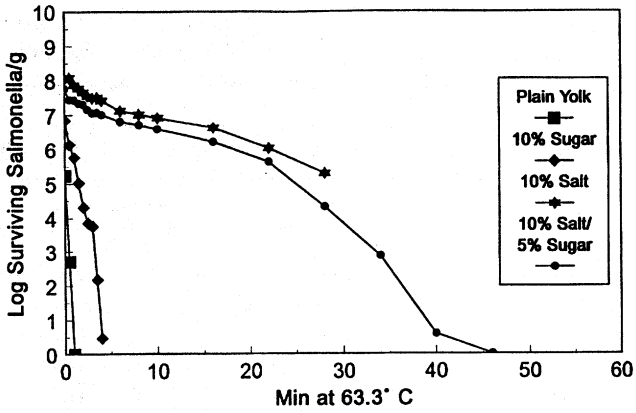

Figure 1. *Survival of* Salmonella *during heating at 63.3°C in plain yolk and yolk containing 10% salt, 10% sugar, and 10% salt plus 5% sugar.*

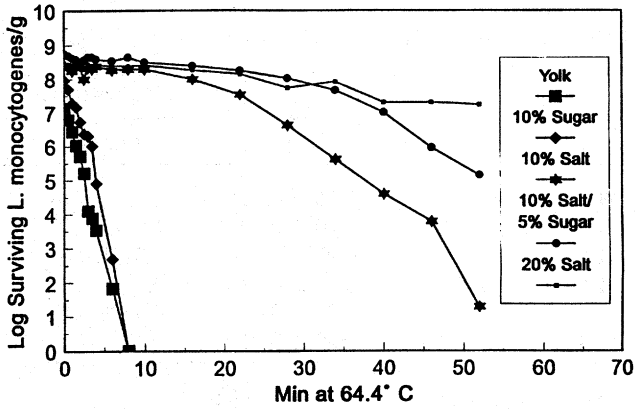

Figure 2. *Survival of* Listeria monocytogenes *during heating at 64.4°C in plain yolk and yolk containing 10% salt, 10% sugar, 20% salt, and 10% salt plus 5% sugar.*

The thermal resistance of each organism in each product for a range of temperatures is shown in Tables 4 and 5. Addition of 10% sugar increased D-values less than 2-fold in most cases, whereas addition of 10% salt caused a measurable lag and increases in D-values of 10- to 50-fold or more. This effect may be due, at least partially, to the greater depressant effect that salt has on a_w of egg yolk (Table 6). Low a_w (0.753) was more protective for *L. monocytogenes* than for *Salmonella*. The D-value for *Salmonella* in 20% salt is actually slightly lower than in 10% salt at two temperatures, possibly reflecting a toxic effect of high salt concentration. The z-values were higher in the 10% salt plus 5% sugar and 20% salt products (9.6 and 8.3°C, respectively) than in the 10% salt product (4.6°C). For *L. monocytogenes*, on the other hand, heating at 64.4 to 66.7°C was 4 to 6 times less lethal in 20% salt than in 10% salt. The z-values for *L. monocytogenes* did not correlate with a_w; for plain yolk, yolk with 10% salt, and yolk with 20% salt, z-values were 6.7, 5.4, and 7.4°C respectively. Garibaldi et al. *(8)* determined the effect of 10% salt and 10% sugar on thermal resistance of *S. typhimurium* TM-1 at 60°C. The D-value was increased from 0.4 min for plain yolk to 4.0 for yolk plus 10% sugar and 5.1 for yolk plus 10% salt. This 10-fold increase in heat resistance with the addition of 10% sugar was not observed in the present study; an explanation may be in differences between the time of inoculation and time of heating or in the strains used. Yen et al. *(27)* inoculated uncured ground pork and pork containing 2% NaCl with a nine-strain mixture of *L. monocytogenes* and heated to 60°C; in the uncured pork, a 5.46-log-unit reduction occurred, whereas in the salted pork only a 3.86-log-unit reduction in CFU/g was observed. Sumner et al. *(25)* studied the heat resistance of *S. typhimurium* and *L. monocytogenes* in sucrose solutions of varying a_w. At 65.6°C, the D-value for *S. typhimurium* was 0.29 min at a_w 0.98 and 4.8 min at a_w 0.89. For *L. monocytogenes* at 65.6°C, the D-value increased from 0.36 min at a_w 0.98 to 3.8 min at a_w 0.90. Of particular interest was an increase in z-value from 7.6°C at a_w 0.98 to 12.9°C at a_w 0.90. Notermans et al. *(19)* showed that two strains of *L. monocytogenes* adapted to 25% sucrose in liquid whole egg and grew during 7 days storage at 4°C.

Current minimum pasteurization processes are 3.5 min at 142°F (61.1°C) for egg yolk, 146°F (63.3°C) for yolk with 10% salt, 10% sugar, or 10% salt plus 5% sugar, and 148°F (64.4°C) for yolk with 20% salt *(1)*. The results of this study indicate that the processes for yolk and yolk with 10% sugar are adequate for destruction of *Salmonella*, but suggest that the current minimum pasteurization processes might permit survival in 10% or 20% salted products if *Salmonella* were initially present at high levels (Table 7). Garibaldi *(7)* suggested the addition of acetic acid for salted yolk intended for salad dressing and mayonnaise production to insure freedom from *Salmonella* contamination. Survival of *L. monocytogenes* could occur

TABLE 4. *Thermal resistance of* Salmonella *spp. in egg yolk products containing sugar (sucrose) and salt (NaCl)*

Heating menstruum	D-value (min) at indicated temperature (°C)				
	61.1	63.3	64.4	65.5	66.7
Plain yolk	0.57 ± 0.05	0.20 ± 0.01	---[a]	---	---
Yolk + 10% sugar	0.74 ± 0.10	0.72 ± 0.20	0.20 ± 0.05	---	---
Yolk + 10% salt	ND[b]	11.50 ± 3.09	6.44 ± 1.31	3.85 ± 0.38	2.07 ± 0.35
Yolk + 10% salt + 5% sugar	ND	8.13 ± 2.38	6.09 ± 3.02	4.37 ± 1.50	3.67 ± 1.15
Yolk + 20% salt	ND	ND	4.60 ± 1.61	3.09 ± 0.60	2.43 ± 0.71

[a] Organisms died during come-up time.
[b] ND, not done.

TABLE 5. *Thermal resistance of* L. monocytogenes *in egg yolk products containing sugar (sucrose) and salt (NaCl)*

Heating menstruum	D-value (min) at indicated temperature (°C)				
	61.1	63.3	64.4	65.5	66.7
Plain yolk	1.41 ± 0.37	0.81 ± 0.07	0.44 ± 0.04	ND[a]	ND
Yolk + 10% sugar	2.05 ± 0.21	1.05 ± 0.22	0.97 ± 0.18	0.63 ± 0.16	0.46 ± 0.04
Yolk + 10% salt	ND	10.5 ± 2.8	6.11 ± 1.85	4.30 ± 1.17	2.39 ± 0.09
		T_L: 14.8[b]	T_L: 16.8	T_L: 8.76	T_L: 5.86
Yolk + 10% salt + 5% sugar	ND	21.3 ± 3.9	8.26 ± 0.67	4.64 ± 0.70	4.58 ± 0.64
		T_L: 30.4	T_L: 21.5	T_L: 19.4	T_L: 3.19
Yolk + 20% salt	ND	ND	27.3 ± 4.6	15.7 ± 2.8	13.3 ± 2.8
			T_L: 10.5	T_L: 14.7	T_L: 12.6

[a] ND, not done.
[b] T_L, lag time (min) before reduction in viable numbers occurred.

TABLE 6. *Water activity (a_w) of egg yolk and egg yolk products containing added salt and/or sugar at 22°C*

Egg yolk product	a_w
Plain yolk	0.989
Yolk with 10% sugar	0.978
Yolk with 10% salt	0.865
Yolk with 10% salt + 5% sugar	0.859
Yolk with 20% salt	0.753

TABLE 7. *Calculated destruction of* Salmonella *spp. and* L. monocytogenes *obtainable under current minimum pasteurization standards (log_{10}-unit reduction in 3.5 min at specified temperature)*

Product	Temperature (°C)	Log_{10} reduction *Salmonella*	Log_{10} reduction *L. monocytogenes*
Egg yolk	61.1	6.14	2.48
Egg yolk + 10% sugar	63.3	4.86	3.33
Egg yolk + 10% salt	63.3	0.30	0.21[a]
Egg yolk + 10% salt + 5% sugar	63.3	0.43	0.05[a]
Egg yolk + 20% salt	64.4	0.76	0.08[a]

[a] Estimated from the overall D-value for the survivor curve. Lag times before killing begins is greater than 3.5 min for these products.

under current standards, depending on the level of contamination. When a lag occurs before initiation of destruction, process calculations must take into account both the lag and the inactivation measured by the D-value.

Accurate estimates of populations of *L. monocytogenes* in egg products will be necessary to evaluate the adequacy of pasteurization. Leasor and Foegeding *(13)* isolated *Listeria* spp. from 36% of the 42 commercial egg samples examined. Two of these samples contained *L. monocytogenes*; the remainder were *L. innocua*. Population levels in the fresh samples were estimated to be well below 100 CFU/g, based on the populations in frozen samples. In an initial survey by the Agricultural Marketing Service designed to detect the presence of *Listeria* species in unpasteurized and pasteurized egg products, 2.38% of unpasteurized and 0.15% of pasteurized samples were positive for *L. monocytogenes*; 29.8% of unpasteurized and 2.63% of pasteurized samples were positive for *L. innocua*, and 0.58% of unpasteurized and 0.08% of pasteurized samples were positive for *L. murrayi* (I. Sterling, personal communication). A subsequent enumeration study of 372 samples found 53 positive for *L. monocytogenes*, of which 31 contained less than 0.3 CFU/g; three whole egg samples contained over 110 CFU/g. Presumably this was the result of environmental contamination in the plant. Production of *Listeria*-free egg products will require attention to sanitation practices as well as pasteurization parameters.

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